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RESEARCH ARTICLE

Induction of Chlorophyll Mutations through Physical and Chemical Mutagenesis in Cowpea [*Vigna unguiculata* (L.) Walp.]

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Abstract

Cowpea variety Pusa komal was treated with γ- rays, EMS and combination of gamma rays with EMS to obtain spectrum and frequency of chlorophyll mutations in M_2 generation in the present investigation. The individual treatment of EMS was found more efficient than gamma rays to induce chlorophyll mutants. A progressive increase in mutation frequency of chlorophyll mutations was observed with increasing doses. Four different types of induced chlorophyll mutants such as, albino, xantha, chlorina and viridis were observed. The highest frequency of chlorophyll mutants (2.00%) was reported at dose of 200Gy + 0.2% EMS. A dose dependent increase in the spectrum and frequency of chlorophyll mutations was observed whether mutagens were applied singly or in combination.

Introduction:-

Cowpea [Vigna unguiculata(L).Walp.] is grown as a food crop by small scale farmers in many regions of the world. Though native to West Africa, this legume has become part of the diet of about 110 million people (Osondu, 1997). In India, cowpea is cultivated in Rajasthan, Gujarat, Punjab, Maharashtra and Tamilnadu. About 22 varieties of cowpea have been recommended for commercial cultivation in different states and territories. In India the estimated area of cultivation is about 5.0 lac hectare with maximum 1.5lac ha area in Tamilnadu where 29000 tonnes cowpea is produced. Mutation breeding has been widely used as a potent method of enhancing variability for crop improvement (Subuthi et al., 1991). Mutagenesis is accomplished by chemical or physical treatments followed by selection for heritable changes of specific genotypes, and this method has been used successfully in the genetic improvement of crop plants. Nuclear gene mutations or extra chromosomal mutations might result in chlorophyll deficient mutations. These chlorophyll mutations are considered as the most dependable indices for evaluating the efficiency of different mutagens in inducing the genetic variability for crop improvement and are also used as genetic markers in basic and applied research. The occurrence of chlorophyll mutations after treatments with physical and chemical mutagens have been reported in several crops (Swaminathan et al., 1962; Solanki, 2005). In the present study, attempt was made to understand comparative response of physical and chemical mutagens on cowpea, with a view to determine the suitable mutagen and effective treatment dose causing genotypic differences so that the frequency of induced chlorophyll mutations in M_2 generation can be observed.

Materials and methods:-

Cowpea variety Pusa Komal was taken for experimentation on mutagenic effect in cowpea. Pusa komal (P85-2/ P426) was released in 1986 by IARI-RS, Karnal and besides higher yield, this variety had early flowering, bore good quality pods and resistance to bacterial blight. With a view to create new variability in this variety, the dry and dormant seeds of cowpea variety Pusa komal were treated with gamma rays, EMS and their combination treatments for conducting present study. The mutagen treatments were given following the standard procedures.

Gamma rays produce electro-magnetic types of radiation that induces mutation through ionization. When a biological material is irradiated, a gamma rays photon hits an orbital electron of the atom, the electron get excited and in turn ejected out leaving behind a positively charge atom. The ejected electron has tremendous energy and is capable of causing further ionization along its path leading to mutations through change in nucleotide sequences. Two hundred well filled healthy seeds packed in moist germination paper having 10% moisture content were taken for each treatment of gamma rays irradiation in the gamma chamber at 100Gy, 200Gy, 300Gy, 400Gy and 500Gy doses. The gamma ray irradiation as physical mutagen was obtained from a 2000 curie CO^{60} gamma cell with a dose rate of 2500 rads per minute installed in the division of nuclear research laboratory (NRL), IARI, New Delhi.

For EMS treatment alone, 200 healthy seeds each were pre-soaked in distilled water for 8hours at room temperature. Thereafter the pre-soaked seeds were treated with 0.1%, 0.2% and 0.3% freshly prepared EMS solution for 8 hours. For combination treatments of gamma rays and EMS, 200 seeds each were first irradiated with gamma rays at 100 Gy, 200 Gy, 300 Gy, 400Gy and 500Gy doses of gamma rays and then treated with 0.2% EMS solution. After EMS treatment, the treated seeds were washed thoroughly for 1hour in running tap water to eliminate the residual effect of the chemical.

After mutagenic treatment, the seeds were sown immediately in the field along with their respective controls at the oil seed research farm of JV College, Baraut to raise the M_1 generation. The experiment was conducted in randomized block design (RBD) with three replications. The population was maintained at row to row spacing of 50cm and plant to plant spacing of 20cm. All the recommended cultural practices were adopted for raising a good crop. The M_2 seedlings were screened from 15^{th} to 20^{th} day to record the various chlorophyll mutants periodically.

Results and Discussions:-

Leaf colour mutations are most frequently observed mutation in both spontaneous and induced mutant populations, and often used as an indicator of mutagenic effects and efficiency of various mutagens**.** Chlorophyll development seems to be controlled by many genes located on several chromosomes, which could be adjacent to centromere and proximal segment of chromosomes (Swaminathan, 1964). Chlorophyll mutations provide one of the most dependable indices for evaluations of genetic effects of mutagenic treatments and have been reported in various pulse crops by several workers (Pandey and Dhanasekar, 2004; Kumar et al., 2011). On the seedling basis of M_2 generation, progressive increase in the frequency of chlorophyll mutation was observed with increase in all mutagenic dose or concentration. The chlorophyll mutatnts may be categorised as albino, xantha, viridis or chlorina. The leaves of albino mutants are white in colour, due to absence of all the pigments leading to the death of the plants at 10-15 days after germination. The leaves turned yellow in colour in xantha due to absence of xanthophylls. Viridis mutants show leaf margin more segregated as compared to control. Young leaves are pale green in chlorina during maturity time. Such mutations are observed in almost all the mutagenic treatments.

A wider range of occurrence of chlorophyll mutations was observed when cowpea variety Pusa Komal was treated with different doses of gamma rays and EMS alone and combination treatment of gamma rays with EMS. Almost all the treatments produced either type of mutants in different frequencies (Table-1, Fig.1). As 600 progenies were evaluated under each of the 14 treatments and control, a total of 99 mutants were observed indicating approx. 1.2% mutagen occurrence. The highest frequency of chlorophyll mutations (2.0%) was observed at 200Gy + 0.2%EMS treatment whereas it was lowest (0.50%) with 100Gy gamma ray treatment.

Among all the mutagen treatments, gamma rays and EMS produced almost equal frequency of mutants when applied alone but the mutagenic efficiency was increased when used in combinations. Among gamma rays doses, 300Gy was effective for producing maximum number of mutants with more frequency of chlorina and xantha mutants that albino and virdis type of mutants. Among EMS doses, 0.2% and 0.3% were equally effective but all types of mutants were observed at 0.2% EMS dose. At 0.3% EMS dose, more frequency of virdis mutants was observed. In combination treatments of gamma rays and EMS, higher frequency of mutants was observed with 200Gy gamma rays with 0.2% EMS followed by 400Gy gamma rays with 0.2% EMS dose.

The perusal of results with respect to types of mutants indicated that all types of chlorophyll mutants occurred after mutagenic treatments. Chlorina mutants were more frequent followed by virdis whereas xantha and albino were observed in similar frequencies. The efficacy of doses indicated maximum production of chlorina and xantha mutants when gamma rays and EMS were applied in combination whereas albino mutants were observed in maximum frequency when treated with EMS alone. The occurrence of virdis mutants was observed in all three types of treatments whether gamma rays and EMS alone or in combination. The chemical mutagen EMS was observed more efficient in production of mutants by Bhattacharya, 2003 and Shah et al., 2006 who attributed it to change in genes needed for chlorophyll development. In present study, higher frequency of chlorophyll mutation at gamma irradiation with moderate doses was observed and it can be assumed that the saturation point for mutagenic effect reaches even at lower or moderate doses in the highly mutable genotypes and further increase in dose does not add to the mutation frequency. With increase in dose beyond a point, the strong mutagens become more lethal than the higher doses of relatively weaker mutagens. The present findings of differential effect of physical and chemical mutagens in inducing chlorophyll mutations are in agreement with earlier report of Mohanasundram et al., 1998.

The combination treatments of gamma rays with EMS enhanced the frequency of chlorophyll mutations (Gautam and Mittal, 1998). In the present investigation no dose dependent increase in the frequency and spectrum of chlorophyll mutations was observed as the lower and higher doses of mutagens produced higher frequency of chlorophyll mutations in EMS and gamma rays. Although occurrence of chlorophyll mutants was observed in all mutagenic treatments, the pre-dominant occurrence of viridis mutant was observed in all mutagenic treatments. The reason for the appearance of greater number of viridis may be attributed to involvement of polygenes in the chlorophyll formation (Ambarkar et al., 2005; Apte et al., 2006). It was also noticed that that viridis was most common mutant whereas albino was least mutant in the present study. Similar observations were noticed in most of the leguminous crops (Chary and Bhalla, 1988; Prakash and Shambulingappa, 1999; Ramya et al, 2014). There was a clear parallelism between the frequency of chlorophyll mutations depending on the mutagens and doses. The frequency of chlorophyll mutations was almost linearly dose dependent in case of gamma rays, EMS as well as their combined treatments.

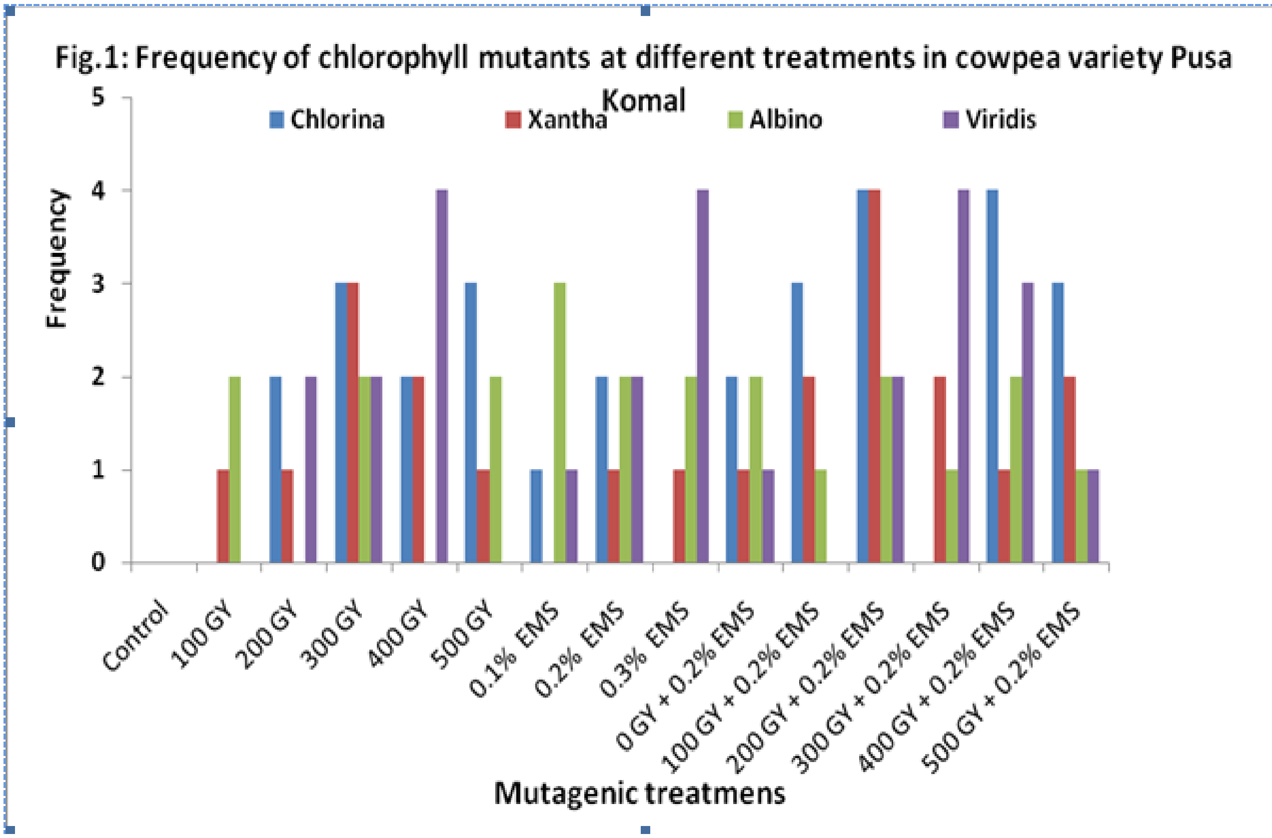

Table1: Frequency of induced chlorophyll mutants in M_2 generation in cowpea variety Pusa Komal:-

Treatment /Doses	No. of M_2 Progenies	Frequency Types of mutations				Total mutants in M_2 generation
		Chlorina	Xantha	Albino	Viridis	
Control	600	0.00	0.00	0.00	0.00	0.00
100 GY	600	0.00	0.16	0.33	0.00	0.50
200 GY	600	0.33	0.16	0.00	0.33	0.83
300 GY	600	0.50	0.50	0.33	0.33	1.66
400 GY	600	0.33	0.33	0.00	0.66	1.33
500 GY	600	0.50	0.16	0.33	0.00	1.00
0.1% EMS	600	0.16	0.00	0.50	0.16	0.83
0.2% EMS	600	0.33	0.16	0.33	0.33	1.16
0.3% EMS	600	0.00	0.16	0.33	0.66	1.16
0 GY + 0.2% EMS	600	0.33	0.16	0.33	0.16	1.00
100 GY + 0.2% EMS	600	0.50	0.33	0.16	0.00	1.00
200 GY + 0.2% EMS	600	0.66	0.66	0.33	0.33	2.00
300 GY + 0.2% EMS	600	0.00	0.33	0.16	0.66	1.16
400 GY + 0.2% EMS	600	0.66	0.16	0.33	0.50	1.66
500 GY + 0.2% EMS	600	0.50	0.33	0.16	0.16	1.16

Conclusion:-

The chlorophyll mutations produced after mutagenic treatments indicate the efficacy of mutagenic treatments either in isolation or in combination. The frequency of such mutants may be helpful in deciding the lethal dose limits for optimising the mutagenic treatments. Although most of the chlorophyll mutants do not have economic importance, the mutagenic effects observed at maturity and their occurrence in next generations may lead to creation of newer plant types for further utilization in crop improvement programmes.

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References:-

1. **Ambarkar, A.S., Harer P.N. and Kulkarni, R.V., (2005)**. Radio sensitivity and visible mutations in chickpea (Cicer arietinum L.). **Adv. Plant Sci.** 18(2): 559-563.
2. **Apte, U. B., Jadhav, B. B. and Nawale, S. R., (2006).** Efficiency and effectiveness of physical and chemical mutagens in cowpea (Vigna unguiculata (L.)Walp). **Journal of Arid Legumes.** 3(1):115-118.
3. **Bhattacharya, C., (2003).** Effect of ethyl methane sulphonate on carnation (Dianthus caryophyllus L.).**Environ. Ecol.,** 21(2): 301-305.
4. **Chary, N.S. and Bhalla, J.K., (1988**). Mutagenic effectiveness and efficiency of gamma rays and EMS on pigeon pea(Cajanus cajan L.Millsp.). **J. Cytol. & Genet**., 23:190-193.
5. **Gautam, A.S. and Mittal, R.K., (1998).** Induced mutations in black gram (Vigna mungo L. Hepper). **Crop Res**, 16(3):344-348.
6. **Kumar, S.T., Thangavel, P. and Anandan, A., (2011**). Studies on induced micro-mutations in cowpea (Vigna unguiculata (L.) Walp.). **Adv.in Pl. Scien.,** 24(1):307-311.
7. **Mohanasundram, M., Thamburaj, S. and Natarajan, S., (1998).** Observations on chlorophyll mutants in vegetable cowpea (Vigna unguiculata (L.) Walp) induced by gamma rays. **South Indian Horticulture.** 46(3/6):243-246.
8. **Osondu, P.O., (1997).** Advances in cowpea research. **Biotechnology and development Monitor,** 33**:** 1012.
9. **Pandey, R.N. and Dhanasekar, P. (2004).** Radiation induced mutant in cowpea. **Journal of Arid Legumes.** 1(1):1-4.
10. **Prakash, B.G. and Shambulingappa, K.G., (1999).**Estimation of chlorophyll and viable mutation under M_2 generation in rice bean (Vigna umbelullata L. Thumb). **Karnataka J. Agr. Sci**., 12(1-4): 58.
11. **Ramya, Nallathambi B. G. and Ganesh Ram S., (2014).** The effect of mutagens on M_1 population of black gram (Vigna mungo L. Hepper). **African Journal of Biotechnology,** 13(8), pp. 951-956
12. **Shah T.M., Mirza J.I., Muhammed A. H. and Babar M. A., (2006).**Induced genetic variability in chickpea (Cicer arietinum L.) I. Frequency and spectrum of chlorophyll mutations. **Pak. J. Bot**., 38(4):1217-1226.
13. **Solanki, I.S., (2005).** Isolation of macro mutations and mutagenic effectiveness and efficiency in lentil (Lens culinaris Medik.).**Indian J. Genet.,** 65: 264-268.
14. **Subuthi, P.K., Mohapatra, B.K. and Sinha, S.K., (1991).**Use of pollen traits for early detection of induced micro mutations in wheat. **Ind. J. Genet**.**,** 5(1):101-111.
15. **Swaminathan, M.S., (1964).** A comparison of mutation induction in diploids and polyploids. In The use of induced mutations in plant breeding. Rad. Mut. **Organ. FAO/IAEA Vienna,** 619-641.
16. **Swaminathan, M.S., Chopra, V.L. and Bhaskaran, S., (1962).**Chromosome aberrations frequency and spectrum of mutations induced by EMS in barley. **Ind. J. Genet,** 22:192-207.